SUPPLEMENTARY INFORMATION

A multimodal digital microfluidic testing platform for antibody-producing cell lines.

Jeremy T. Lant[1,2], Jurgen Frasheri[1,2,3], Taehong Kwon[4], Camille Tsang[2], Betty Li[1,2], Sheldon Decombe[1,2], Alexandros A. Sklavounos[1,2], Samin Akbari[4], and Aaron Wheeler[1,2,3]

[1] Department of Chemistry, University of Toronto, Toronto, Ontario, Canada

[2]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada

[3]Institute of Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada

[4]Sartorius Stedim North America Inc., Marlborough, MA, USA

SUPPLEMENTAL FIGURES

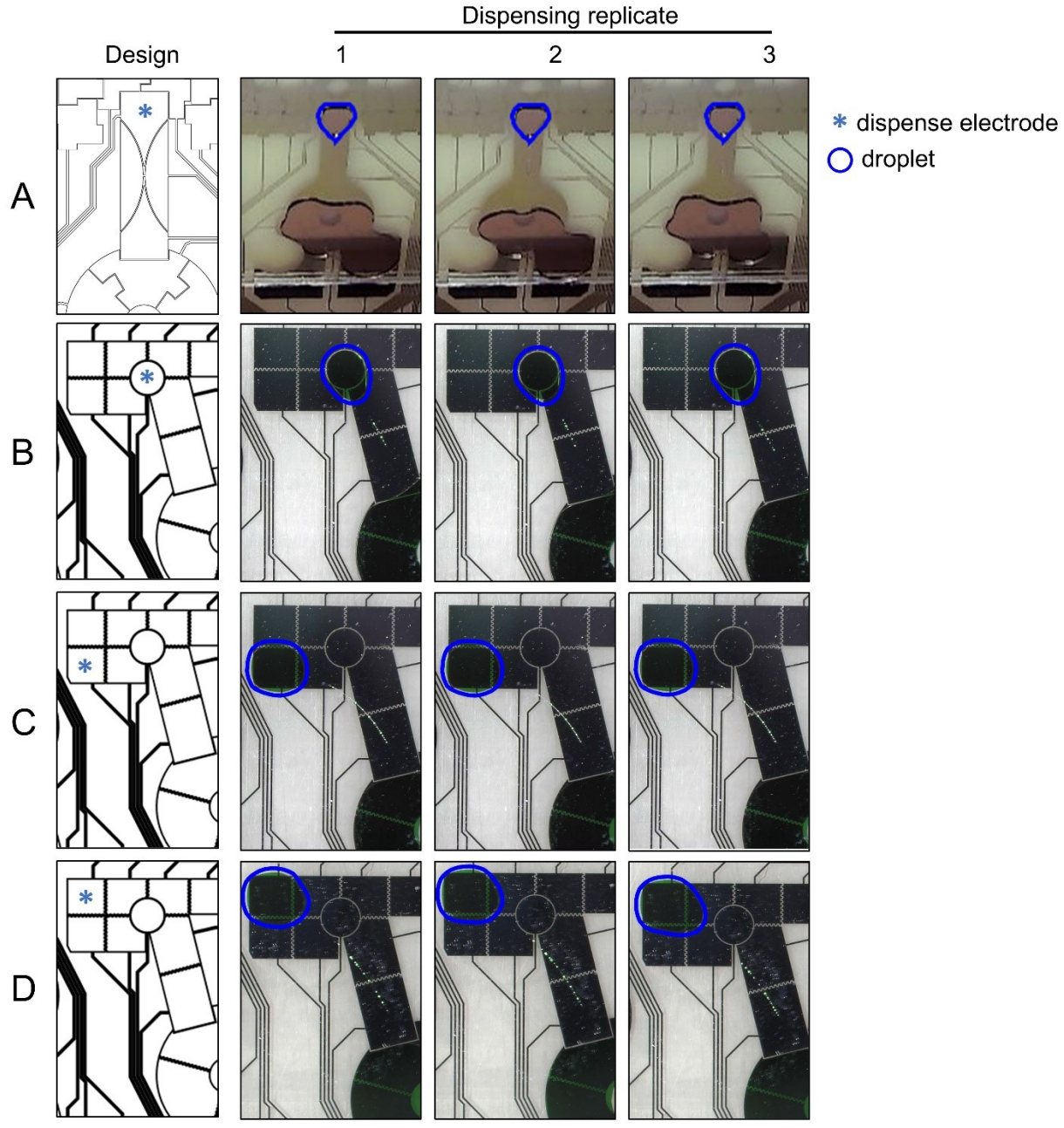

Figure S1. *Cellspot* dispensing electrode optimization. Cartoons (left) of different electrode designs and configurations tested for dispensing (left), and photographs (right) of three replicate droplets dispensed in each device. The top row is an early version of a *Cellspot* device featuring a "dumbbell" electrode design (A; adapted from *Micromachines* 13, 2022, 284). The three rows on the bottom (B-D) feature the final device design, operated with different capture electrodes. Blue stars in the cartoons indicate the electrodes used to capture the dispensed droplet, and blue traces in the photos indicate the droplet perimeters.

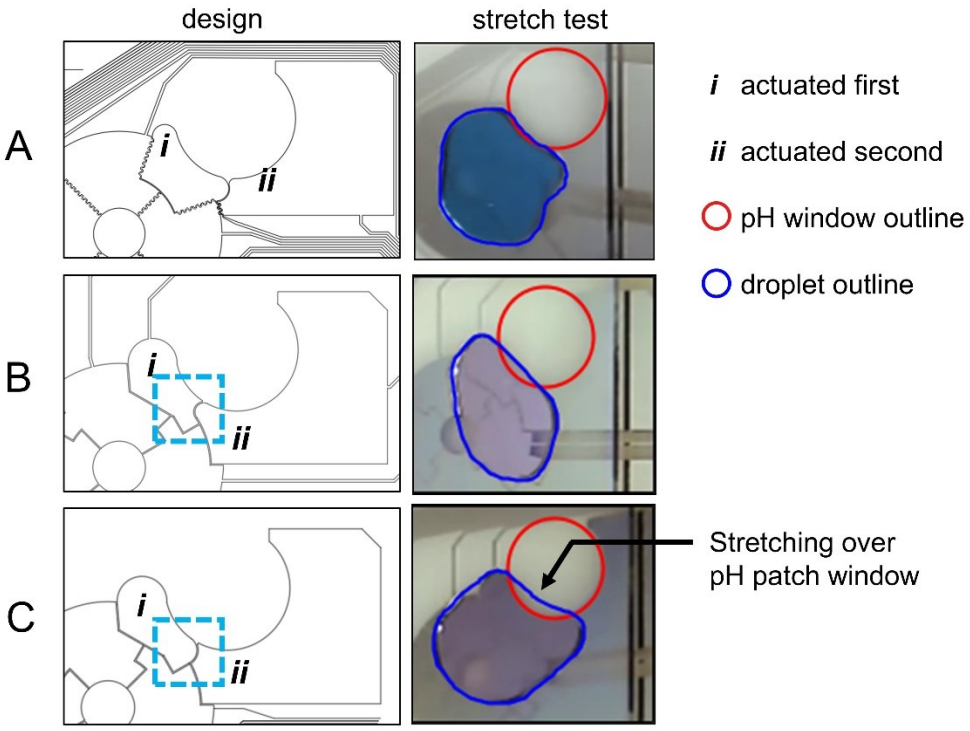

Figure S2. *Cellspot* loading electrode optimization for pH sensor patch. Cartoons (left) and photographs (right) of droplet 'stretch tests' on early versions of the design (A,B) and the final version of the design (C). pH sensing patches are not adhered to devices in these images. In the final implementation, stretching of droplets onto adhered pH sensing patches initiated absorption of sample into the patch. Droplet stretching over the pH sensing window was accomplished by actuating the electrode labelled "i" first, then actuating both electrodes "i" and "ii". The label on the photo in the bottom row indicates droplet stretching into pH sensor patch window region. Blue traces indicate droplet perimeters, red traces indicate pH window perimeters, and dashed light blue boxes indicate the region where designs B and C differ.

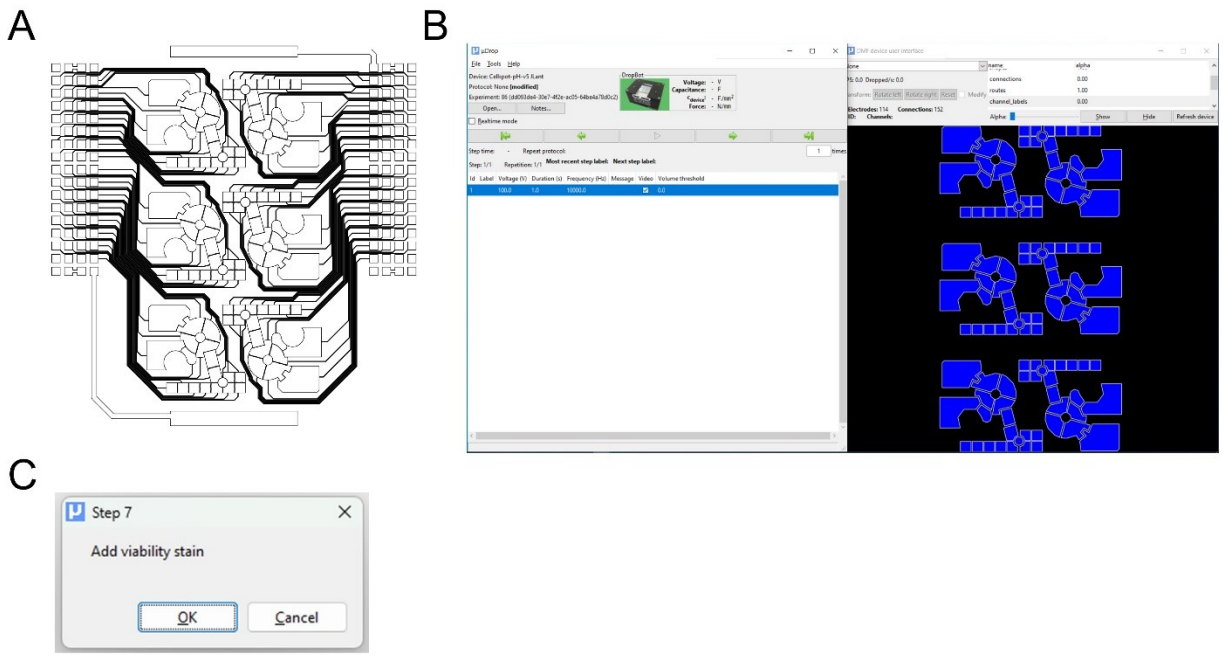

Figure S3. Full *Cellspot* device design and software implementation. (a) CAD drawing of the *Cellspot* DMF device with six intercalated *Cellspot* features. (b) Screenshot of Cellspot design in the MicroDrop user interface. (c) Screenshot of a user prompt message for reagent addition.

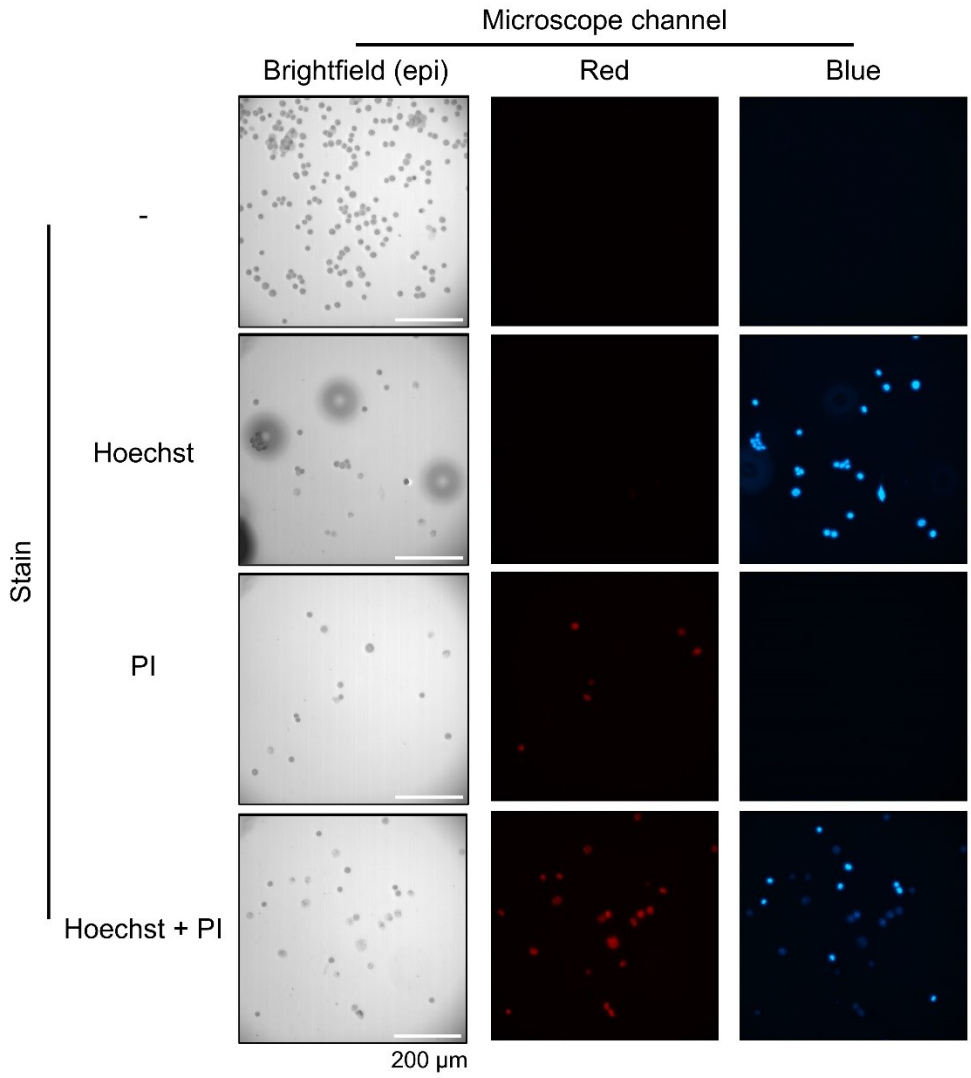

Figure S4. Live/dead cell stain compatibility. Epifluorescence microscopy images of CHO cell suspension stained with PBS control (-), Hoescht 33342 (Hoescht), Propidium iodide (PI), or both Hoescht and Propidium iodide. Images were captured from droplets on a *Cellspot* DMF device.

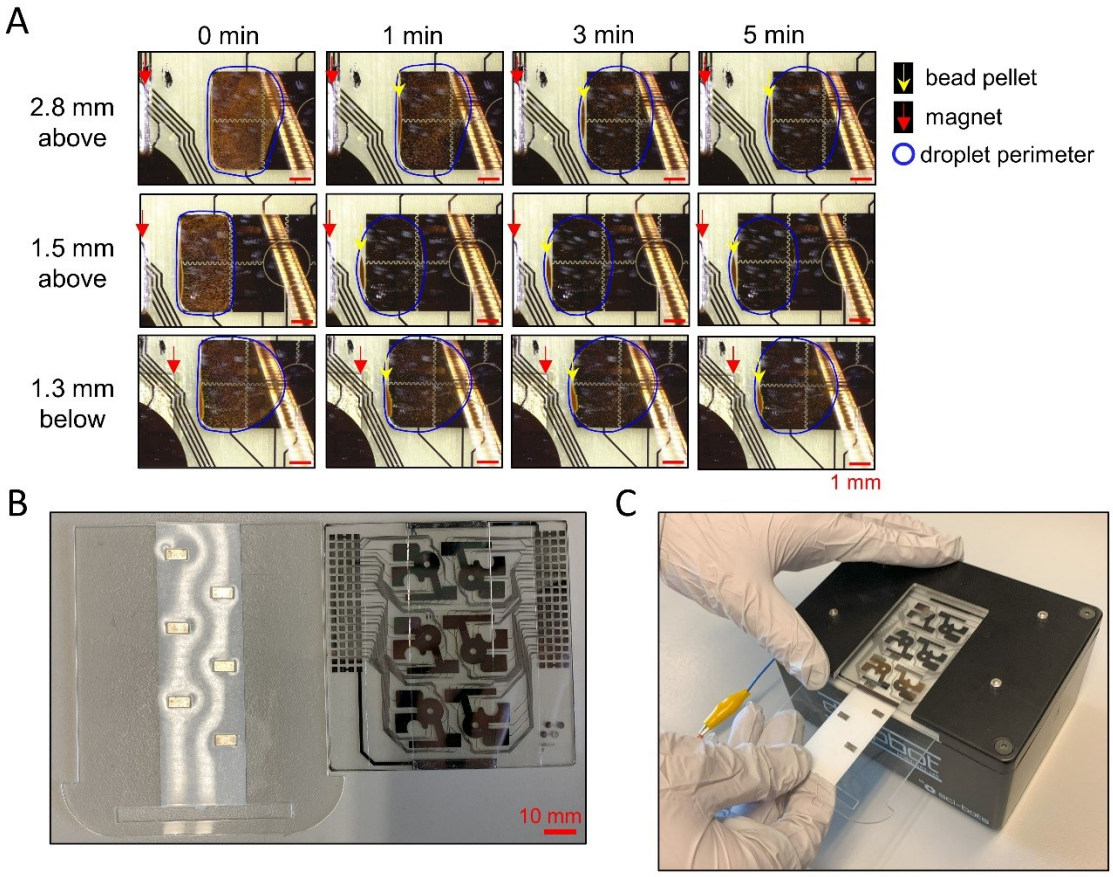

Figure S5. Magnetic plate optimization. (a) Time series images (columns, left-to-right) of magnet bead pelleting on a *Cellspot* DMF device with magnet positioned at various locations horizontally to the left of the droplet (2.8 mm, 1.5 mm, or 1.3 mm) above or below the device (rows). Red arrows indicate magnet position. Yellow arrows indicate magnetic bead pellet at droplet edge. Blue trace indicates droplet perimeter. (b) Photograph of a 6x magnetic plate (left) next to a *Cellspot* DMF device (right). (c) Photograph demonstrating sliding of a magnetic plate under a *Cellspot* device while in a Dropbot system (Sci-Bots).

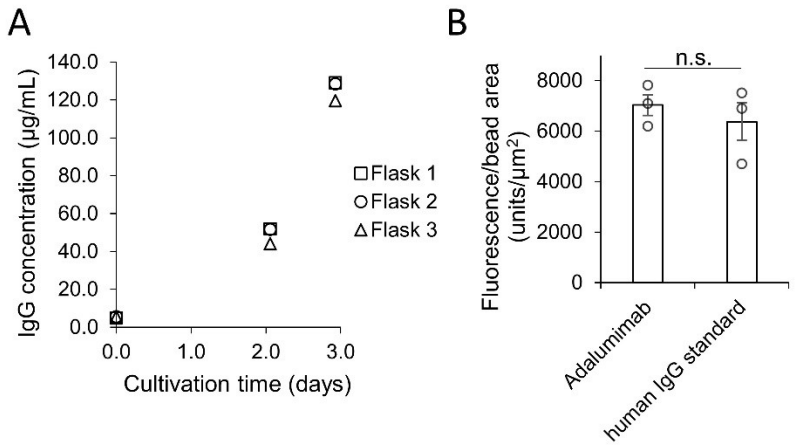

Figure S6. FLISA assay bench-marking. A) Plot of IgG production in CHO DG44-Adalumimab cell line (measured using Sartorius Octet BLI instrument calibrated with human IgG standard) as a function of cultivation time. Markers (square, circle, triangle) indicate independent measurements from different flasks of cells. (b) Calibration antibody comparison for DMF FLISA assay. On-DMF FLISA assays were completed using either purified Adalimumab or generic human IgG standard at 0.4 g/L concentration diluted in cell culture medium. Bars represent means of three independent replicates. Open circles represent underlying sample replicate data and error bars represent standard deviation. The means were not significantly different (n.s.) according to a single factor ANOVA test ($\alpha = 0.05$).

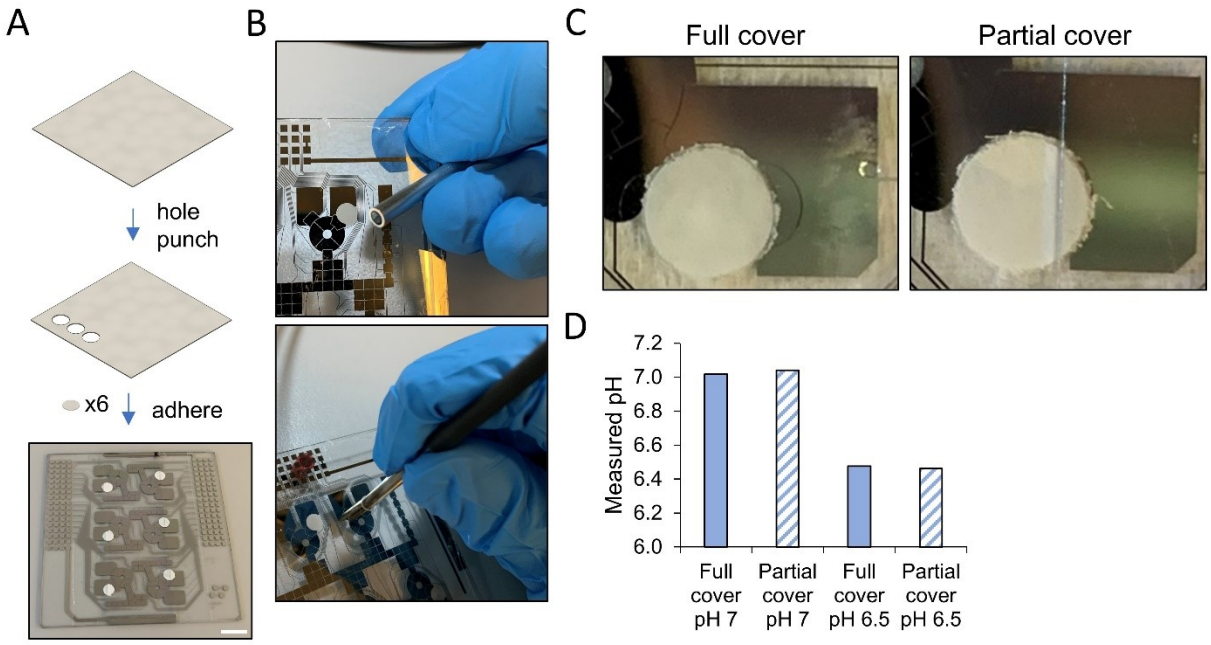

Figure S7. Media pH assay demonstration and testing. (a) Cartoon and photographs demonstrating how a PreSens optical pH sensor is brought close to a *Cellspot* DMF device, and (b) photographs illustrating how the sensor is aligned with a PreSens pH patch that had been pre-adhered to the device bottom plate (right). (c) Photographs and (d) Plots of pH measured using the PreSens optical pH meter in a *Cellspot* DMF device with top plate fully (left photo, solid bars) or partially (right photo, hatched bars) covering the adhered pH patch.

SUPPLEMENTAL METHODS

Viability assay analysis. Images of Hoescht- or propidium iodide-stained cells were imported to Fiji/ImageJ and analyzed using Macro 1 (see appendix). The macro output includes counts from the Hoescht and PI stained images. Cell viability was calculated using the formula:

$$cell\ viability\ \% = \left(\frac{count_{Hoescht}}{count_{Hoescht} + count_{PI}}\right) \times 100$$

For manual counts (Fig. 2D-F in the main text), images were imported into Fiji/ImageJ and counted by eye using the multipoint labelling tool.

FLISA assay analysis. Images of Dynabeads protein A with bound fluorescent antibody (Alexa Fluor^{Tm} 488) were imported to Fiji/ImageJ and analyzed using Macro 2 (see appendix). The macro output includes integrated density, area, and "mean" (integrated density/area) values corresponding to individual beads or clusters of beads in the image. For each image, the mean intensity/area and standard deviation were calculated in Microsoft Excel. The mean intensity/area value from the 0 µg/mL IgG control replicates was subtracted from all other values, and below zero values were corrected to zero. A standard curve was fit to data from experiments with increasing concentrations of a human IgG standard (see Fig. 4E in the main text). Several curve fitting models were tested with a graphing calculator and a Gompertz model fit with the following formula was selected as it had the lowest residual standard error of the models tested.

Gompertz model:

$$y = 4460.474exp(-8.821\exp(-11.79x))$$

Where y is the observed bead fluorescence/area (units/µm^2) and x is the human IgG concentration in the sample. To convert observed fluorescence/area values from our experiments to IgG concentrations, we expressed the curve formula in terms of x and inputted the observed fluorescence/area values (y) from our experiments:

$$x = -(ln(-\frac{\ln\left(\frac{y}{4460.474}\right)}{8.821}}{11.79})$$

APPENDIX – FIJI/IMAGEJ MACROS

Analyses described herein were done in Fiji/ImageJ version 1.54d with Java version 1.8.0_172 (64-bit) on a Windows 11 operating system. Adjustments to the macro may be required to run the macro on different operating systems. Lines in the macros beginning with "//" denote commentary on the lines of code below.

Macro 1. Viability assay. The following macro was used for analyses shown in figures 2D-F, and 6B.

```
//Open the Hoescht stained image. Replace "IMAGE" with your file location.
open("IMAGE");
//Threshold to capture area of entire Hoescht-stained droplet. Should be adjusted to your image series using the Image → Adjust → Threshold… function.
setThreshold(1800, 65535);
run("Convert to Mask");
//Clean up the mask image before analysis.
run("Fill Holes");
run("Despeckle");
run("Despeckle");
run("Despeckle");
run("Watershed");
//Use analyze particles to make ROI annotation around the droplet area. Change the lower area cutoff as necessary to exclude any thresholded objects besides the Hoescht-stained droplet.
run("Analyze Particles...", "size=500000-Infinity display exclude clear summarize add composite");
//Re-open original Hoescht image
open("IMAGE ");
//Remove spurious pixels with despeckle function. This prevents the next functions from miscounting noise as a cell.
run("Despeckle");
//Overlay droplet ROI from before
```

```
roiManager("Show None");
roiManager("Show All");
roiManager("Select", 0);
//Find maxima within the ROI area. Adjust the prominence setting as necessary for you image sequence.
run("Find Maxima...", "prominence=10000 strict output=List");
//Save results.
saveAs("NAME");
//Clear Hoescht results before counting next image.
run("Clear Results");
//Open the propidium iodide-stained image. Replace “IMAGE” with your file location.
open("IMAGE");
//All other steps are essentially the same as done for Hoescht count. Extra despeckle steps or adjusted prominence setting may be required depending on microscope setting compatibility with PI staining.
run("Despeckle");
run("Despeckle");
run("Despeckle");
roiManager("Show None");
roiManager("Show All");
roiManager("Select", 0);
run("Find Maxima...", "prominence=3000 strict output=List");
//Save results.
saveAs("NAME");
```

Macro 2. FLISA assay. The following macro was used for analyses shown in figures 4C,D, and 6E.

```
//Open fluorescent bead images. Replace “IMAGES” with your file location.
File.openSequence("IMAGES");
//Subtract background fluorescence using rolling circle function, radius 20. Radius should be adjusted depending on image magnification and bead size.
run("Subtract Background...", "rolling=20 stack");
//Threshold using the IsoData method, convert to black on white mask image.
setAutoThreshold("IsoData dark");
```

```
setOption("BlackBackground", false);

run("Convert to Mask", "method=IsoData background=Dark");

//Remove noise with despeckle function

run("Despeckle", "stack");

run("Despeckle", "stack");

run("Despeckle", "stack");

//Separate large clusters with watershed function.

run("Watershed", "stack");

//Generate annotations (ROIs) with analyze particles function. Size cutoffs should be adjusted according to bead size and image magnification.

run("Analyze Particles...", "size=10-3000 circularity=0-1 display exclude clear summarize add composite stack");

//Clear results window.

run("Clear Results");

//Open original fluorescent bead images again. Replace “IMAGES” with your file location.

File.openSequence("IMAGES");

//Subtract background fluorescence using rolling circle function, radius 20. Radius should be adjusted depending on image magnification and bead size.

run("Subtract Background...", "rolling=50 stack");

//Toggle ROIs to overlay on fluorescent image.

roiManager("Show None");

roiManager("Show All");

//Set measurements to include desired parameters. “Mean” gives the intensity per unit area for each ROI, which is used for fluorescence values.

run("Set Measurements...", "area mean standard integrated shape redirect=None decimal=3");

roiManager("Measure");

//After running the macro, the fluorescence values can be found in the results window and the image numbers associated with each ROI (if using a series of images) can be found in the ROI manager. Save both for analysis.
```